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Characterization of polymorphic microsatellite DNA markers in the black-tailed godwit (*Limosa limosa:* Aves)

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Abstract

We isolated and tested 16 microsatellite loci in black-tailed godwits from the Netherlands (*Limosa limosa limosa*), and from Australasia (subspecies *melanuroides*). One locus was monomorphic, two loci had null-alleles and one was significantly heterozygote deficient. The remaining 12 polymorphic loci had on average 7.9 alleles (range 5–11) and the mean expected heterozygosity was 0.69. No significant linkage disequilibrium between the loci was observed and all loci were autosomal. Fourteen loci were successfully cross-amplified in bar-tailed godwit (*Limosa lapponica*).

Keywords: black-tailed godwit, ground-nesting shorebird, *Limosa limosa*, microsatellite, population genetics

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The black-tailed godwit (*Limosa limosa*) is a philopatric ground-nesting shorebird, found throughout Eurasia in grassland, wetland, and marine intertidal habitats. It breeds from Iceland to eastern Russia and overwinters in Europe, Africa, the Middle East and Australasia. It has three subspecies *L. l. islandica*, *L. l. limosa* and *L. l. melanuroides* (Höglund *et al*. 2009). Recent survey data suggest that global populations of black-tailed godwit may have declined by 30% over the last 15 years (BirdLife International 2008). The species is qualified as Near-Threatened according the IUCN Red List of Threatened Species. Agricultural intensification and habitat loss because of wetland drainage might explain these declines (IUCN 2008). To manage effectively the conservation programmes, information on spatial population structuring, genetic diversity, population size, parentage and dispersal patterns is needed. In the context of a long-term study on metapopulation dynamics of black-tailed godwits in agricultural landscapes (Kentie *et al*. 2008), we developed microsatellite markers.

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Genomic DNA was extracted from the blood of two godwits from the Dutch *limosa* population (stored in 95% ethanol at −80 °C, band numbers 3477284 and 3477285), using DNeasy Tissue Kits (Qiagen). A DNA library was constructed following Hamilton *et al*. (1999). DNA was digested using three blunt-end restriction enzymes (*Nhe*I, *Rde*I, *Hae*III; New England Biolabs), and ligated to SNX linkers to enable PCR amplification of inserts. Amplification was followed by hybridization to biotinylated oligonucleotides with GT and AC motifs. Biotinylated DNA fragments were captured using Dynabeads (Dynal). Enriched DNA was recovered using PCR. Amplified enriched DNA was ligated into a PCR 2.1 cloning vector, and plasmids were transformed to competent cells using TA Cloning kit (Invitrogen). A total of 576 positive clones were isolated on membranes and a second hybridization with biotinylated oligonucleotides using NRET Phototope Star Detection detected 207 clones with inserts. Positive clones were sequenced with forward and reverse M13 primers using BigDye Terminator version 3.1 (Applied Biosystems) and an ABI 3100 automated capillary sequencer (Applied Biosystems).

Table 1 Characterization of 15 polymorphic microsatellite DNA markers in the Eurasian black-tailed godwit breeding population in the Netherlands (*Limosa limosa limosa*: Aves)

Locus GenBank accession no.	Primer sequence (5′–3′)	Repeat	T_A	MgCl	Dye	n_a	N	Size range	H_O	H_E	Null allele
LIM3	F: AGACGACTCTATGACGCTGTG	$(CA)_{25}$	54	2.5	VIC	11	20	203–225	0.81	0.83	—
FJ652578	R: CGGGATGAGACGCAACAC						7		0.86	0.81	
LIM5	F: ACTGCTGCTTCCAAATGACA	$(GA)_{10}AA(GA)_2$	54	2.5	NED	5	22	218–232	0.41	0.40	—
FJ652579	R: TCTCCCCTCCATCTGAAAAG						8		0.63	0.51	
LIM8	F: CTGAAGTGATCAGGCAAGGTG	$(CA)_{12}$	57	2.5	FAM	8	22	194–212	0.77	0.75	—
FJ652580	R: TGTGGAGGAAGGAGGCTTG						8		0.63	0.82	
LIM10	F: GCAAAGAATCCAAAGCCCAG	$(CA)_9$	57	2.5	FAM	9	20	242–263	0.80	0.73	—
FJ652581	R: TCCTGTTACTTCCAGTGGCTG						8		0.75	0.87	
LIM11	F: TCCCTGGTCAAATTGAGTTGT	$(CA)_{12}$	54	2.5	VIC	9	22	212–244	0.86	0.82	—
FJ652582	R: GGATTACAGCCGGCACTG						8		0.75	0.77	
LIM12a	F: GGTTCTCTTTGGGCTGTCTG	$(CA)_{10}$	54	2.5	NED	10	22	465–493	0.82	0.85	—
FJ652583	R: AGGAAACTCATCTGGGGTCTG						8		0.88	0.81	
LIM12b	F: GCCAAAATATTTGACAGACCCCAG	$(CA)_{11}$	57	2.5	FAM	7	22	231–245	*0.59*	*0.78*	—
FJ652584	R: GAGTTTCCCAGCACTTTGCCC						8		0.75	0.75	
LIM14	F: TCAAACAGGTGGCAGCAG	$(CA)_9(AG)_2(AC)_8AG(AC)_2$	57	2.5	VIC	15	19	68–128	*0.53*	*0.83*	0.16
FJ652585	R: CTGCAAACCCCCACCAAG						8		*0.37*	*0.87*	
LIM22	F: TGAAGCCAAAATGTTCCAAA	$(CA)_9$	54	2.5	VIC	6	20	261–273	0.45	0.46	—
FJ652586	R: TGCCTTTTTGTTCCACACTG						8		0.76	0.54	
LIM24	F: GTCGTTTTCTATTGCACAGGCAAG	$(GA)_{14}$	57	2.5	VIC	9	22	230–252	0.90	0.78	—
FJ652587	R: GATGTTTTGTAGAGCTGAAGATGA						8		0.88	0.88	
LIM25	F: TGACACCAGACAGTGTTGCAT	$(CA)_4AAAA(CA)_8$	57	2.5	FAM	5	22	171–181	0.36	0.35	—
FJ652588	R: CCGACTTTATTTGGTTTTCCAG						7		0.43	0.38	
LIM26	F: TCAGGTGGACGTACAGCAC	$(CA)_{11}$	57	2.5	FAM	10	22	378–396	0.80	0.82	—
FJ652589	R: GCAAGTCAAGGGGTGGAAC						8		1.00	0.85	
LIM30	F: ACCTTAGTACATGGGGAACAG	$(CA)_{10}$	57	2.5	FAM	5	21	322–336	0.52	0.56	—
FJ652590	R: TGAAGGCATATCTGGGGATGTC						6		0.17	0.17	
LIM32	F: TCAGACGTCGATCACCTGAG	$(CA)_9CC(CA)_6$	54	2.5	VIC	13	19	148–178	*0.53*	*0.85*	0.17
FJ652591	R: TGAAACTATAAATCCTGCGGG						7		*0.43*	*0.95*	
LIM33	F: CTTCCAAGGGAGGGTGAC	$(CA)_{16}(A)_5C(A)_6(A)_x$	54	2.5	FAM	8	22	175–191	0.73	0.74	—
FJ652592	R: AGGTTGTTGGGTTGGGTG						8		0.39	0.69	
LIM6*	F: TATTCAGCAGAACACACGCAC	$(AC)_5GC(AC)_6$	54	2.5	FAM	1	—				
FJ652593	R: ACTGGTATTCTTTAACCCCGACTG										

In addition, the markers were tested in the Australasian subspecies *melanuroides*.

T_a, annealling temperature; dye, universal dye-labelled M13 tail; n_a, number of alleles; N, number of individuals successfully genotyped per subspecies, with *limosa* in the upper line and *melanuroides* in the lower line; H_O, observed heterozygosity; H_E, expected heterozygosity; values in italic indicate that loci significantly deviated from Hardy–Weinberg equilibrium. Null allele, frequency of null alleles calculated by Brookfield equation 1, as given in MICRO-CHECKER (Van Oosterhout *et al.* 2004).

*Characterized only in *Limosa lapponica*, see Table 2.

Sequences were assembled and edited using ChromasPro 1.33 and MEGA 3.1 (Kumar *et al*. 2004).

Twenty-four sequenced clones had motif repeats with unique flanking sequences, for which primers were designed using Fast PCR (Kalendar 2009) and Primer3 (Rozen & Skaletsky 2000). PCR was carried out on a Mastercycler epgradient S (Eppendorf). The volume of PCR reaction mixture was 12.5 μL, which contained 1× PCR buffer (10 mM Tris–HCL pH 8.3, 50 mM KCl, 2.5 mM $MgCl_2$, 0.01% gelatin), 0.7 pmol dNTP (Invitrogen), 0.75 pmol forward primer (Invitrogen), 2.5 pmol reverse primer (Invitrogen), and 2.5 pmol universal dye-labelled M13 (TGTAAAACGACGGCCAGT) tail (6-FAM, NED, VIC; Applied Biosystems), 0.25 U Taq (Invitrogen) and 1 μL DNA template. PCR conditions included an initial denaturation step at 94 °C for 5 min, 36 cycles of denaturation at 94 °C for 30 s, primer annealing at 54 or 57 °C for 30 s and primer extension at 72 °C for 30 s. A final step at 72 °C for 5 min was used to complete primer extension. Fragment analysis was run on the ABI 3100. Alleles were sized using 500LIZ size standard (GeneScan™); allele sizes were assigned with GeneMapper 3.7 (Applied Biosystems). Consistent PCR products were obtained for 18 primer pairs, which were screened using *limosa* specimen from the Netherlands (n = 22). In addition, we screened *melanuroides* specimen from Vietnam and Northwest Australia (n = 8). Tests for linkage disequilibrium were run on all data (n = 30). Furthermore, primers were tested on two populations of bar-tailed godwits (*Limosa lapponica*, n = 37).

In black-tailed godwits, two of the 18 markers yielded inconsistent allele calls. Sixteen loci were tested for polymorphism, the presence of null alleles, allele drop-out, and stuttering using MICRO-CHECKER version 2.2.3 (Van Oosterhout *et al*. 2004). Hardy–Weinberg equilibrium tests were run in Arlequin version 3.11 (Excoffier & Schneider 2005). Linkage equilibrium was tested using GENEPOP (http://genepop.curtin.edu.au/ (Raymond & Rousset 1995) and FSTAT (Goudet 2001) applying sequential Bonferroni correction (Rice 1989). Sex-linkage was tested by molecular sexing using the primers 2602F (O. Haddrath) and 2718R (Fridolfsson & Ellegren 1999). To assess allele scoring problems, scoring was repeated independently by KT. Of the 16 loci, one was monomorphic, two loci had null-alleles and one was significantly deficient in heterozygotes (Table 1). The remaining 12 polymorphic loci had on average 7.9 alleles (range 5–11) and a mean expected heterozygosity of 0.69. No significant linkage disequilibrium was observed (P > 0.001). For each locus, we detected heterozygous females indicating that the loci were autosomal. The scoring consistency was 99%.

Fourteen loci were successfully cross-amplified in the bar-tailed godwit (Table 2). Two loci were monomorphic and a third locus was nearly monomorphic (H_O = 0.05). One locus had null-alleles. The remaining 10 polymorphic loci had on average 5.5 alleles (range 3–9) and a mean expected heterozygosity of 0.50.

Table 2 Cross amplification of 14 microsatellite DNA markers in bar-tailed godwit (*Limosa lapponica*: Aves)

Locus*	n_a	N	Size range	H_O	H_E	Null allele
LIM3	6	16	193–207	0.80	0.81	—
	6	16		0.69	0.75	—
LIM5	5	16	218–230	0.31	0.29	—
	4	21		0.29	0.26	–
LIM6	3	16	230–238	0.13	0.12	—
	2	17		0.06	0.06	—
LIM8	5	16	200–210	0.43	0.53	—
	3	17		0.47	0.57	—
LIM10	4	16	248–260	0.25	0.51	0.16
	5	20		0.60	0.58	—
LIM12a	4	16	469–477	0.80	0.63	—
	5	18		0.67	0.57	—
LIM12b	2	16	229–241	0.19	0.18	—
	5	20		0.25	0.28	—
LIM22	1	16	264–266	n/a	n/a	n/a
	2	20		0.05	0.05	—
LIM25	3	16	173–177	0.44	0.38	—
	3	19		0.47	0.44	—
LIM26	9	16	382–406	0.60	0.82	0.11
	9	20		0.65	0.82	—
LIM30	8	16	321–337	0.94	0.87	—
	9	21		0.86	0.83	—
LIM32	6	16	150–168	0.31	0.81	0.27
	9	20		0.53	0.79	0.14
Monomorphic						
LIM11	1	16	234			n/a
		20				n/a
LIM24	1	8	234			n/a

N, number of individuals genotyped with samples from the Netherlands in the upper line and samples from Australia, New Zealand and Alaska in the lower line; n_a, number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity; loci that significantly deviated from Hardy–Weinberg equilibrium are in italics. Null allele, frequency of null alleles calculated by Brookfield equation 1, as given in MICRO-CHECKER (Van Oosterhout *et al*. 2004).

*LIM14 and LIM33 were not screened as test amplifications indicated at primer binding at multiple sites.

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Isolation and characterization of the first eight microsatellite loci in *Gammarus fossarum* (Crustacea, Amphipoda) and cross-amplification in *Gammarus pulex* and *Gammarus orinos*

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Abstract

We characterized the first microsatellite markers for *Gammarus fossarum*. Eight loci gave satisfactory amplification patterns in two stream populations (Southern France) with number of alleles ranging from 2 to 10 and expected heterozygosity from 0.076 to 0.857. We performed cross-amplification in two closely related gammarid species, *Gammarus pulex* and *Gammarus orinos*. Among the eight tested microsatellite loci, four correctly amplified in *G. pulex* and three in *G. orinos*.

Keywords: cross-species amplification, *Gammarus*, microsatellite-enriched library, microsatellites

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Because of some unclear boundaries between species, Gammarids phylogeny have focused the interest of many scientists (Kolding & Simonsen 1983; Meyran *et al.* 1997; Hou *et al.* 2007). However, knowledge on their population genetic structure (reviewed in Hogg *et al.* 2000) is still scarce. The present work characterizing the first microsatellites in *Gammarus fossarum* and studying their

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